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Torque teno virus (TTV) Probe qPCR Kit (with Internal Control)

Product Number: DTK589NC

Shipping and Storage

Transport at low temperature; store at -20°C with a shelf life of one year. The positive control shall be placed separately to avoid contaminating other reagents.

Component

Component	50T
2× Multiplex Probe qPCR Mix	650μL
DEPC-H_2O	1mL
Fluorescent template diluent	1mL
Human TTV qPCR Primer-Probe Mix (with Internal Reference)	390μL
Human TTV qPCR Negative Control (with Internal Reference)	100μL
Human TTV qPCR Positive Control (with Internal Reference) (1.00E+08 copies/μL)	50μL

Note: The positive control contains the target gene fragment of human TTV and the target gene fragment of eukaryotic internal reference control; the negative control contains the target gene fragment of eukaryotic internal reference control.

Features

This kit is used for the detection of human Torque Teno Virus (TTV). Developed based on the principle of probe-based real-time fluorescent quantitative PCR, this human TTV detection kit has the following features:

1. Ready-to-use; users only need to provide sample DNA templates.
2. Contains internal reference control.
3. Provided with positive control to facilitate the identification of false-negative samples.
4. High specificity: Primers are designed targeting the highly conserved regions of human TTV DNA sequences, and will not cross-react with DNA from other biological samples.
5. This product is sufficient for 50 probe-based qPCR reactions with a 25μL reaction system.
6. This product is for research use only.

Protocol

1. DNA extraction (sample preparation area)

Extract and purify sample DNA by self-selected methods. This kit is compatible with most nucleic acid extraction kits on the market.

2. Dilution of Standard Curve Samples (Sample Preparation Area)

(Due to the high concentration of the positive control, the following dilution operations must be performed in an independent area to avoid contamination of samples or other components of this kit.)

2.1. Label 6 centrifuge tubes as Tube 7, 6, 5, 4, 3, and 2 respectively.

2.2. Add 45μL of fluorescent template diluent to each tube with filter pipette tips (filter tips are recommended throughout the procedure, the same below).

2.3. Add 5μL of 1.00E+08 copiesμL positive control (provided with the kit) to Tube 7, mix thoroughly by vortexing for 1 minute to obtain a standard curve sample at 1.00E+07 copies/μL. Place on ice for later use.

2.4. Change the pipette tip, add 5μL of 1.00E+07 copies/μL positive control (obtained from the previous dilution) to Tube 6, mix thoroughly by vortexing for 1 minute to obtain a standard curve sample at 1.00E+06 copies/μL. Place on ice for later use.

2.5. Change the pipette tip, add 5μL of 1.00E+06 copies/μL positive control (obtained from the previous dilution) to Tube 5, mix thoroughly by vortexing for 1 minute to obtain a standard curve sample at 1.00E+05 copies/μL. Place on ice for later use.

2.6. Repeat the above operations until standard curve samples with 6 dilution gradients are obtained. Place on ice for later use.

If no standard curve is required and only qualitative detection is performed, dilute the positive control to 1.00E+05 copies/μL.

3. Reagent Preparation (Reagent Preparation Area)

Prepare sufficient qPCR tubes (sample tubes, negative control tubes, positive control tubes), and add the following components to each qPCR tube respectively.

Component	N sample tubes to be tested	qPCR negative control	qPCR positive control
2 ×Multiplex Probe qPCR Mix	12.5μL each	12.5μL	12.5μL
Human TTV qPCR Primer-Probe Mix (with Internal Reference)	7.5μL each	7.5μL	7.55μL

Transfer all tubes to the template addition area.

4. Add Template (Template Add Area)

Add 5μL of template to each qPCR tube in the following order: human TTV qPCR negative control, test sample template, human TTV qPCR positive control. Centrifuge for 30 seconds and perform the amplification reaction immediately.

5. Amplification reaction (amplification and product analysis area)

Place the qPCR tubes in the corresponding positions of the sample slot of the qPCR instrument for amplification. The amplification program is as follows:

Step	Temperature	Time
Pre denaturation	95℃	3min
qPCR reaction 45 cycles	95℃	15sec
	60℃	60sec
Signal channel	Collect fluorescence signals by selecting FAM/HEX channels simultaneously	

6. Result Analysis

6.1. Quality Control: If any of the following conditions is not met, the test result is considered invalid: Negative control: No FAM fluorescent signal detected, HEX fluorescent signal detected, and Ct value of the HEX channel ≤ 30.0; Positive control (1.00E+05 copies/μL): Both FAM and HEX fluorescent signals detected, and both Ct values ≤ 30.0.

6.2. If a standard curve is constructed, plot the standard curve with the logarithmic value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the logarithmic value of sample DNA concentration from the standard curve based on the Ct value of the test sample, and derive the actual concentration.

6.3. If no standard curve is constructed, judge the results according to the following criteria:

Sample test results:

Ct value of FAM channel ≤ 38, Ct value of HEX channel ≤ 30, with obvious exponential amplification: positive result, the sample contains human TTV;

Ct value of FAM channel ≥ 40 or no Ct value detected, and Ct value of HEX channel ≤ 30: negative result, the sample does not contain human TTV;

Ct value of FAM channel ranges from 38 to 40, and Ct value of HEX channel ≤ 30: the sample shall be retested. If the Ct value remains within 38–40 with obvious exponential amplification after retest, judge as positive; otherwise negative;

Ct value of HEX channel > 30.0 or no amplification curve: re-sampling and re-detection are required.